Spectral Assignments and Reference Data

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Structural determination of abutilins A and B, new flavonoids from *Abutilon pakistanicum*, by 1D and 2D NMR spectroscopy

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Two new flavonoids, abutilin A and B, were isolated from the chloroform soluble fraction of *Abutilon pakistanicum* and their structures assigned from ^{1}H and ^{13}C NMR spectra, DEPT and by 2D COSY, HMQC and HMBC experiments. Ferulic acid (3), (*E*)-cinnamic acid (4), 5-hydroxy-4′,6,7,8-tetramethoxyflavone (5), kaempferol (6), luteolin (7) and luteolin 7-*O*-β-D-glucopyranoside (8) have also been reported from this species.

Keywords: NMR; 1D/2D NMR; flavonoids; *Abutilon pakistanicum*; Malvaceae; abutilin A; abutilin B

Introduction

The genus *Abutilon*, which belongs to the family Malvaceae, comprises 150 species and is distributed in subtropical regions of Asia and other parts of the world in the form of perennial herbs and shrubs and rarely as small trees. Generally the leaves, roots and stems of *Abutilon* species contain considerable amounts of mucilage because of which these are used for the treatment of rheumatism and as demulcents and diuretics.[1–3] The ethnopharmacological and chemotaxonomic importance of the genus *Abutilon* led us to investigate the chemical constituents of one of its species, namely *Abutilon pakistanicum*. The literature survey revealed that only one steroid and three triterpenes have so far been reported from this species.[4–6] We now report the isolation and structural elucidation of two new flavonoids named abutilins A (**1**) and B (**2**) based on spectroscopic techniques including 1D and 2D NMR. In addition, ferulic acid (**3**),[7] (*E*)-cinnamic acid (**4**),[8] 5-hydroxy-,4′,6,7,8-tetramethoxyflavone (**5**),[9] kaempferol (**6**),[10,11] luteolin (**7**)[12] and luteolin 7-*O*-β-D-glucopyranoside (**8**)[13] have also been isolated for the first time from this species.

Results and Discussion

The methanolic extract of the whole plant was divided into *n*-hexane, chloroform, ethyl acetate, *n*-butanol and water soluble fractions. Column chromatography (CC) of the chloroform soluble fraction provided compounds **1–8.** Their structures were established by UV, IR, MS and NMR spectroscopy. Both abutilins A (**1**) and B (**2**) were isolated as yellow amorphous powder, and they gave violet coloration with ferric chloride for a phenolic moiety and were recognized as flavonoid glycosides from their positive tests with Molish and Shinoda reagents.[14]

The UV spectrum of abutilins A (**1**) was characteristic of flavonoidal glycoside showing absorptions at λ_{max} 206, 275 and 315 nm.[15] On addition of $AlCl_3$/HCl, it showed a bathochromic shift of 40 nm, suggesting the presence of chelated hydroxyl group at C-5 of a flavonoid.[16]• On the other hand, addition of NaOAc resulted in a bathochromic shift of 11 nm, suggesting the presence of free hydroxyl group at C-7. The IR spectrum showed the presence of hydroxyl group (3330–3445 cm^{-1}), ester carbonyl (1675 cm^{-1}), conjugated carbonyl (1660 cm^{-1}) and aromatic moieties (1535 and 1500 cm^{-1}). The molecular formula was deduced as $C_{24}H_{24}O_{12}$ through high-resolution electron impact mass spectroscopy (HR-EI-MS) which showed the M^+ peak at *m/z* 504.1263 (calculated for $C_{24}H_{24}O_{12}$, 504.1268). The broadband (BB) and DEPT ^{13}C NMR spectra displayed 24 signals comprising 10 quaternary, 11 methine, 1 methylene and 2 methyl carbons (Table 1).

The ^{1}H NMR spectrum of **1** showed a characteristic low-frequency signal at δ 13.6 assignable to the chelated hydroxyl group at C-5 of a flavonoid. Two *meta*-coupled aromatic protons at δ 6.74 (1H, d, J = 2.1 Hz) and δ 6.81 (1H, d, J = 2.1 Hz) were diagnostic for a C-5 and C-7 oxygenated ring A. The ^{1}H NMR spectrum showed three further aromatic protons of ring B forming an ABX system at δ 7.16 (1H, d, J = 8.8 Hz), 7.71 (1H, dd, J = 8.8, 2.1 Hz) and 7.55 (1H, d, J = 2.1 Hz). The signal of H-3 resonated at δ 6.96 (s, 1H), and one methoxyl group was observed at 3.75 (s, 3H). The presence of an acetylated hexose moiety in β configuration was evident by the signals of an anomeric proton at δ 5.74 (1H, d, J = 7.6 Hz) and a methyl singlet at δ 1.95 (3 H) due to the acetyl moiety. Further signals of oxymethines and oxymethylene of the sugar moiety were observed at δ 4.77 (1 H, m, H-2″), 4.25 (1 H, m, H-3″), 4.14 (1 H, m, H-4″), 4.28 (1 H, m, H-5″), 4.96 (1 H, dd, J = 11.2, 2.6 H-6″ a) and 4.39 (1 H, dd, J = 11.2, 2.8, H-6″ b). EI-MS spectra showed a peak at *m/z* 300 resulting from the loss of aceylated hexose moiety, and further retro Diels–Alder fragments

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Table 1. ^{1}H, ^{13}C NMR and HMBC correlations of compound **1** (C_5D_5N)

Position	^{13}C-NMR	^{1}H-NMR	HMBC
2	163.8	–	–
3	105.1	6.96 (1H, s)	2, 4, 10, 1′
4	182.6	–	–
5	158.5	–	–
6	100.1	6.74 (1H, d, $J = 2.1$ Hz)	5, 7, 8, 10
7	165.9	–	–
8	95.0	6.81 (1H, d, $J = 2.1$ Hz)	6, 7, 9
9	163.0	–	–
10	104.1	–	–
5-OH	–	13.6	–
1′	125.6	–	–
2′	115.9	7.55 (1H, d, $J = 2.1$ Hz)	1′, 3′, 4′
3′	150.8	–	–
4′	150.1	–	–
5′	110.6	7.16 (1H, d, $J = 8.8$ Hz)	3′, 4′, 6′
6′	120.3	7.71 (1H, dd, $J = 8.8, 2.1$ Hz)	1′, 2′, 5′
1″	101.7	5.74 (1H, d, $J = 7.6$ Hz)	4′ 2″, 5″
2″	74.8	4.77 (m)	1″, 2″-O**C**OCH$_3$
3″	76.6	4.25 (m)	1″, 2″, 5″
4″	71.1	4.14 (m)	2″, 5″, 6″
5″	78.2	4.28 (m)	1″, 6″
6″a	63.4	4.96 (1H, dd, $J = 11.2, 2.6$ Hz)	5″
6″b		4.39 (1H, dd, $J = 11.2, 2.8$ Hz)	
2″-O**C**OCH$_3$	170.5	–	–
2″-OCO**CH**$_3$	20.6	1.95 (3H, s)	2″-OCOCH$_3$
3′-OCH$_3$	56.0	3.75 (3H, s)	3′

Table 2. ^{1}H, ^{13}C NMR and HMBC correlations of compound **2** (C_5D_5N)

Position	^{13}C-NMR	^{1}H-NMR	HMBC
2	157.6	–	–
3	134.4	–	–
4	178.7	–	–
5	157.8	–	–
6	99.9	6.67 (1H, d, $J = 2.0$ Hz)	5, 7, 8, 10
7	166.0	–	–
8	94.6	6.65 (1H, d, $J = 2.0$ Hz)	6, 7, 9
9	162.7	–	–
10	105.2	–	–
1′	121.9	–	–
2′, 6′	130.6	8.42 (2H, d, $J = 8.7$ Hz)	2, 3′, 4′, 5′
3′, 5′	116.0	7.21(2H, d, $J = 8.7$ Hz)	2′, 4′, 6′
4′	161.7	–	–
3-OH	–	12.34	–
5-OH	–	13.18	–
1″	104.1	6.21 (1H, d, $J = 7.5$ Hz)	4′, 2″, 5″
2″	76.0	4.21 (m)	1″, 4″
3″	71.3	4.18 (m)	1″, 2″, 5″
4″	78.4	4.82 (m)	2″, 5″
5″a	64.2	4.95 (1H, dd, $J = 9.8, 2.0$ Hz)	4″
5″b		4.38 (1H, dd, $J = 9.8, 4.1$ Hz)	
1‴	167.2	–	–
2‴	114.9	6.48 (1H, d, $J = 15.8$ Hz)	3‴, 4‴
3‴	145.1	7.82 (1H, d, $J = 15.8$ Hz)	1‴, 5‴, 9‴
4‴	126.5	–	–
5‴, 9‴	131.8	7.48 (2H, d, $J = 8.6$ Hz)	4‴, 6‴, 7‴
6‴, 8‴	116.7	7.13 (2H, d, $J = 8.6$ Hz)	4‴, 5‴, 9‴
7‴	161.3	–	–

at *m/z* 152 and *m/z* 148 confirmed the presence of methoxyl and hydroxyl groups in ring B, and two hydroxyl groups in ring A, respectively.

In BB and DEPT (135 and 90) ^{13}C NMR spectra, the signals at δ 163.8, 105.1, 182.6, 163.0 and 104.1 were typical of C-2, C-3, C-4, C-9 and C-10 of a flavonol moiety.[17] The position of methoxyl group was not only authenticated through HMBC but also through nuclear Overhausser effect (NOE) between methoxyl protons at δ 3.75 and H-2′. The ^{1}H and ^{13}C NMR data of the flavonoid skeleton were in complete agreement to those of chrysoeriol.[18] Abutilin A (**1**) is, therefore, the glycoside of chrysoeriol. Acid hydrolysis provided chrysoeriol (mp 330–331 °C) and the sugar moiety, the latter being confirmed as D-glucose through the sign of its optical rotation ($[\alpha]_D$ + 52.8) and comparative thin-layer chromatography (Co-TLC) with an authentic sample. The position of glucose moiety was established at C-4′ by HMBC experiments in which the anomeric proton at δ 5.74 showed ^{3}J correlation with C-4′ at δ 150.1. The low frequency shift of H-2″ allowed us to assign the acetyl moiety to this position. It was confirmed through HMBC experiments in which the ester carbonyl carbon at δ 170.5 showed ^{2}J and ^{3}J correlations with acetyl methyl at δ 1.95 and H-2″ at δ 4.77. This could further be confirmed through ^{1}H–^{1}H COSY, which showed correlation between the anomeric proton and H-2″. The HMBC (Table 1), HMQC and ^{1}H–^{1}H COSY spectra were in complete agreement to the assigned structure of abutilin A (**1**) as 5,7,4′ trihydroxy-3′-methoxy flavone-4′-*O*-β-D-(2″-*O*-acetyl) glucopyranoside (Fig. 1).

The molecular formula of abutilin B (**2**) was deduced from the positive mode high-resolution fast atom bombardment mass spectrometry (HR-FAB-MS), giving a pseudomolecular ion peak $[M + H]^+$ at *m/z* 565.1342 (calculated for 565.1347), consistent with the molecular formula $C_{29}H_{25}O_{12}$. It was further confirmed by the ^{13}C NMR (BB and DEPT) spectra, which showed 29 signals comprising 1 methylene, 16 methine and 12 quaternary carbons (Table 2). The UV spectrum was characteristic of flavonoidal glycoside, showing absorptions at λ_{max} 208, 265 and 321 nm. On addition of $AlCl_3$/HCl, it showed significant bathochromic shifts due to chelated phenolic groups.[16] The IR spectrum displayed the signal for hydroxyl group (3320–3415 cm^{-1}), ester carbonyl (1684 cm^{-1}), conjugated carbonyl (1665 cm^{-1}), olefinic bond (1645 cm^{-1}) and aromatic moieties (1540 and 1505 cm^{-1}).

The ^{1}H NMR spectrum of **2** showed low frequency signals at δ 12.34 and 13.18 assignable to the chelated hydroxyl groups at C-3 and C-5 of a flavonoid moiety. Two *meta*-coupled doublets (J = 2.0 Hz), each integrating for one proton at δ 6.67 and 6.65, were diagnostic for a C-5 and C-7 oxygenated ring A. It further showed the presence of four aromatic protons of *para*-substituted ring B showing AA′XX′ pattern at δ 8.42 (d, J = 8.7 Hz) and 7.21 (d, J = 8.7 Hz), respectively. The presence of (*E*)-*p*-coumaroyl moiety could be inferred from the *trans*-olefinic protons at δ 7.82

Magnetic Resonance in Chemistry

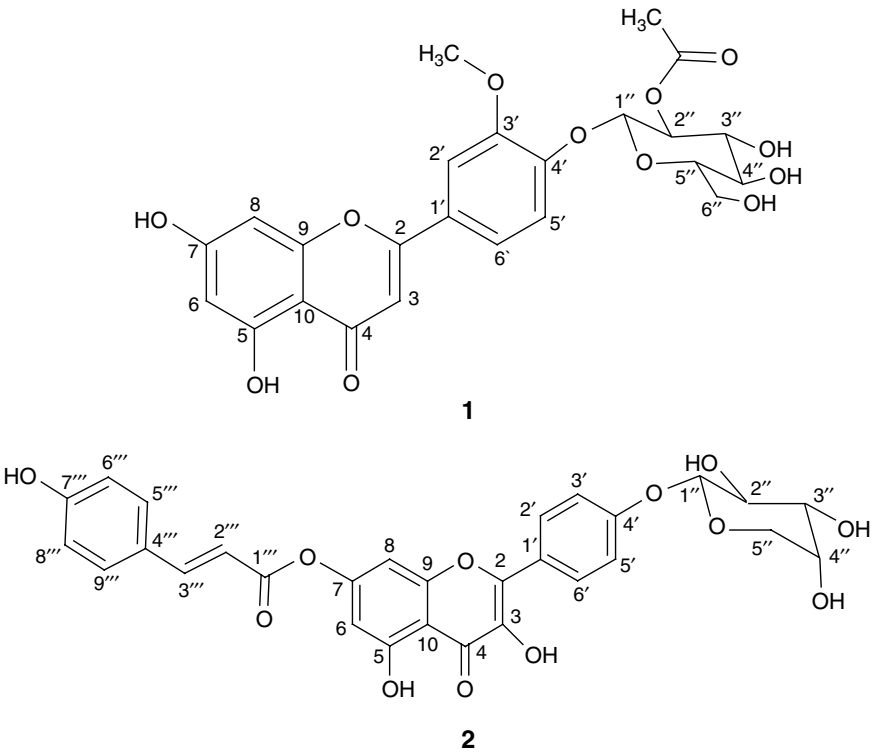

Figure 1. Structures of abutilins A (**1**) and B (**2**).

and δ 6.48 (d, J = 15.8 Hz)[19] as well as protrons of *p*-hydroxy phenyl ring showing another AA′XX′ pattern at 7.48 (d, J = 8.6 Hz) and 7.13 (d, J = 8.6 Hz), respectively. The presence of a pentose moiety was revealed by the signal of anomeric proton at δ 6.21 and further signals of oxymethine and oxymethylene protons at δ 4.21 (m, H-2″), 4.18 (m, H-3″), 4.82 (m, H-4″), 4.95 (dd, J = 9.8, 2.0, H-5″ a) and 4.38 (dd, J = 9.8, 4.1, H-5″ b). The ^{13}C NMR signals at δ 157.6, 134.4, 178.7, 162.7 and 105.2 were typical of C-2, C-3, C-4, C-9 and C-10 of a flavonol moiety. The anomeric carbon was observed at δ 104.1 and further signals of the pentose moiety were observed at δ 76.0 (C-2″), 71.3 (C-3″), 78.4 (C-4″) and 64.2 (C-5″). The ^{13}C NMR spectrum further showed signals of ester carbonyl at δ 167.2 and olefinic carbons at 114.9 and 145.1 along with signals of benzene ring moiety at δ 126.5, 131.8, 116.7 and 161.3.

The key to the structure of abutilin A (**2**) was provided by the acid hydrolysis, which afforded, besides the sugar moiety, a mixture of aglycones identified as (*E*)-*p*-coumaric acid and kaempferol.[10,11] The sugar could be identified as L-arabinose through Co-TLC and sign of its optical rotation $[\alpha]_D^{20}$ + 102.1. The larger coupling constant of the anomeric proton (J = 7.5 Hz) allowed us to assign α configuration to the pentose moiety. This was also confirmed by EI-MS, which showed fragments at *m/z* 432 [M$^+$ − *p*-coumaroyl] and 286 [M$^+$ − *p*-coumaroyl − L-arabinose]. In addition, the presence of retro Diels–Alder fragments at *m/z* 152 and *m/z* 134 confirmed the presence of one hydroxyl and (*E*)-*p*-coumaroyl moiety in ring A. The remaining problem was to locate the point of attachment of the sugar moiety. The HMBC experiments showed ^3J correlation of the anomeric proton at δ 6.21 with C-4′ (161.7), C-3‴ (71.3), C-5″ (64.2) and ^2J correlations with C-2″ (76.0). This allowed us to assign the sugar moiety at C-4′; therefore the (*E*)-*p*-coumaroyl moiety must be at C-7. The important HMBC correlations illustrated in Table 2 are in complete agreement with the assigned structure of abutilin B (**2**), as 7-*O*-(*E*)-*p*-coumaroyl kaempferol 4′-*O*-α-L-arabinopyranoside (Fig. 1).

Experimental

Methods

Column chromatography was carried out using silica gel (230–400 mesh, E. Merck, Darmstadt, Germany). TLC was performed on precoated silica gel G-25-UV$_{254}$ plates (20 × 20 cm, E. Merck, Darmstadt, Germany) and detection was done at 254 nm, and by spraying with ceric sulfate in 10% H_2SO_4. Optical rotations were measured on a JASCO DIP-360 polarimeter. UV spectra were recorded on a Hitachi UV-3200 spectrophotometer. The IR spectra were recorded on Jasco 302-A spectrometer. Mass spectra (EI and HR-EI-MS) were measured in the electron impact mode on Finnigan MAT 12 or MAT 312 spectrometers and ions are given in *m/z* (%). Positive mode HR-FAB-MS was recorded on a Jeol JMS-DA-500 mass spectrometer. Melting points were determined on a Gallenkemp apparatus and are uncorrected.

NMR spectra

The 1D and 2D NMR spectra were recorded on a Bruker AMX-400 spectrometer in C_5D_5N operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts are in ppm (δ), relative to tetramethylsilane as an internal standard, and scalar couplings are reported in hertz. The pulse conditions were as follows: for the ^{1}H NMR spectra, observation frequency 400 MHz, acquisition time (AQ) = 2.732 s, number of scans (NS) = 128, number of dummy scans (DS) = 0, relaxation delay (RD) = 1.0 s, 90° pulse width = 5.40 µs, spectral

width (SW) = 7183.91 Hz, line broadening (LB) = 0.3 Hz and fourier transform (FT) size = 32 768; for the ^{13}C NMR spectrum, observation frequency 100.613 MHz, AQ = 0.654 s, NS = 3000, DS = 2, RD = 1.5 s, SW = 25075.64 Hz, FT size = 32 768, LB = 1.5 Hz; for the DEPT 135 spectrum, observation frequency = 100.632 MHz, AQ = 1.0879476 s, NS = 10 823, DS = 2, RD = 1.5 s, SW = 15060.241 Hz, LB = 1.00 Hz; DEPT 90 spectrum, observation frequency = 100.618 MHz, AQ = 1.0879476 s, NS = 1029, DS = 2, RD = 1.5 s, RG = 32 768, SW = 15060.241 Hz, LB = 1.00 Hz; the COSY 45° spectra were recorded at 400 MHz at SW of 4810 Hz in F_2 3065 and F_1 in the 3082 domain. The AQ was 0.14 s, the spectra were acquired with 1K data points in F_2 with 32 transients, 8 DS and 256 experiments. For the NOESY experiments the SWs of 2904 for F_2 and F_1 domains were recorded. The AQ was 0.15 s, 1025 × 256 data points, 128 transients and 500 ms mixing time. The long range ^{1}H–^{13}C correlations (HMBC) spectra resulted from 1024 × 256 data matrix with 16 scans per t_1 increments. Spectral width of 25 658 Hz in F_1 and 3412 Hz in F_2 domain were used. The AQ was 0.14 s, the delays were set to 3.45 ms ($1/^2J$ (C, H)) and 65 ms (corresponding to an average $1/^nJ$ (C, H) of 7.7 Hz) and the recycle time was 1.44 s. Fourier transform was done on a 2K × 1K data matrix. The HMQC spectra was collected in the t_1 domain in 256 experiments with 2K data points and SWs of 3375 and 20 785 in the F_2 and F_1 dimensions, respectively. The relaxation delay D_1 was set to 1.5 s and D_2 was empirically optimized to 3.5 ms.

Plant material

The whole plant of *A. pakistanicum* Jafri and Ali (8 kg) was collected from Karachi and identified by the Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen (No. 697 KUH) has been deposited.

Extraction and isolation

The freshly collected whole plant material of *A. pakistanicum* (8 Kg) was cut into small pieces and extracted with MeOH (3 × 20 l). The combined methanolic extract was evaporated under reduced pressure to yield a residue (350 g), which was divided into *n*-hexane (65 g), $CHCl_3$ (75 g), EtOAc (35 g), *n*-BuOH (90 g) and water (60 g) soluble fractions. The chloroform soluble fraction (75 g) was subjected to CC over silica gel eluting with *n*-hexane–$CHCl_3$, $CHCl_3$ and $CHCl_3$–MeOH in increasing order of polarity. The fractions that were obtained from *n*-hexane–chloroform (3.5 : 6.5) were combined and rechromatographed over silica gel eluting with *n*-hexane–chloroform (3.8 : 6.2) to afford compound **3** (25 mg) and compound **4** (25 mg). The fractions that were obtained from *n*-hexane–chloroform (1.0 : 9.0) were combined and rechromatographed over silica gel eluting with *n*-hexane–chloroform (1.5 : 8.5) to afford compound **5** (25 mg). The fractions that were obtained from chloroform–methanol (9.0 : 1.0) were combined and rechromatographed over silica gel eluting with chloroform–methanol in increasing order of polarity. The fractions obtained from $CHCl_3$–MeOH (9.5 : 0.5) were subjected to preparative TLC ($CHCl_3$–MeOH, 8.5 : 1.5) to afford compounds **6** (17 mg) and **7** (14 mg), respectively. The fractions which were obtained from $CHCl_3$–MeOH (9.0 : 1.0) were combined and rechromatographed over silica gel eluting with $CHCl_3$–MeOH (9.3 : 0.7) to afford abutilin A (**1**) (18 mg) and compound **8** (25 mg). The fractions that were obtained from $CHCl_3$–MeOH (8.0 : 2.0) were combined and subjected to preparative TLC over silica gel ($CHCl_3$–MeOH; 7.2 : 2.8) to afford abutilin B (**2**) (22 mg).

Compound (**1**): Yellow amorphous powder, UV λ_{max}^{MeOH} (log ε) nm: 206 (2.5), 275 (1.4), 315 (3.8); IR ν_{max}^{KBr} cm^{-1}: 3330–3445 (OH), 1675 (ester CO), 1660 (CO), 1535, 1500 (aromatic moieties); $[\alpha]_D^{25}$ −75.5 (c = 0.1, MeOH); EI-MS (70 e/v) (rel.int %) 504 (15), 300 (100), 285 (55), 272 (24), 257 (15), 229 (36), 162 (28), 152 (35), 148 (50), 133 (35), 114 (21), 1.5 (20). HR-EI-MS *m/z* 504.1263 (calculated for $C_{24}H_{24}O_{12}$, 504.1268). For ^{1}H, ^{13}C NMR and HMBC data see Table 1.

Compound (**2**): Yellow amorphous powder, UV λ_{max}^{MeOH} (log ε) nm: 208 (2.9), 265 (2.2), 321 (4.5); IR ν_{max}^{KBr} cm^{-1}: 3320–3415 (OH), 1684 (ester CO), 1665 (CO), 1645 (C=C), 1540, 1505 (aromatic moieties); $[\alpha]_D^{25}$ −104.5 (c = 0.05, MeOH); EI-MS (70 e/v) (rel.int %) 432 (22), 404 (46), 339 (23), 298 (15), 286 (100), 257 (75), 232 (29), 213 (35), 209 (81), 164 (76), 152 (32), 150 (16), 134 (24) 85 (30), 60 (35). HR-FAB-MS (positive mode) *m/z*: 565.1342 $[M + H]^+$ (calculated for $C_{29}H_{25}O_{12}$, 565.1347); For ^{1}H, ^{13}C NMR and HMBC data see Table 2.

Acid hydrolysis of 1 and 2

Abutilin A (**1**) (8 mg) in 10% HCl was refluxed for 40 min. The cooled reaction extracted with ethyl acetate. The EtOAc fraction crystallized from methanol, and was identified as chrysoeriol (mp 330–331 °C) through comparison of physical and spectral data with those reported in the literature.[18] The aqueous phase was concentrated and the sugar was identified as D-glucose by the sign of its optical rotation ($[\alpha]_D$ + 52.8) and Co-TLC with an authentic sample of D-glucose using the solvent system *n*-BuOH–EtOAc–HOAc–H_2O (12 : 2 : 2 : 2). TLC was run three times in the same direction and spots were visualized with aniline phthalate reagent. It was further confirmed by comparing the retention time of TMS ether with standard sample in GC.[20,21]

Acid hydrolysis of abutilin B (**2**) under similar reaction conditions yielded a mixture of aglycones which could be separated through preparative TLC and identified as kaempferol (mp 277–279 °C) and (*E*)-*p*-coumaric acid (mp 211–213 °C), respectively. The sugar was identified as L-arabinose by Co-TLC using solvent system $CHCl_3$: MeOH (7 : 3) and the sign of its optical rotation ($[\alpha]_D^{20}$ = +102.1).

References

[1] E. Nasir, S. I. Ali, *Flora of West Pakistan*, Fakhri Priniting Press: Karachi, **1972**, pp. 53, 71, 712, 761, 764.
[2] E. Nasir, S. I. Ali, *Flora of West Pakistan*, Fakhri Priniting Press: Karachi, **1979**, pp. 60, 130.
[3] S. R. Baquar, in *Medicinal and Poisonous Plants of Pakistan*, Printas: Karachi, **1989**, p 2.
[4] Z. Ahmed, S. N. H. Kazmi, A. Malik, *J. Nat. Prod.* **1990**, *53*, 1342.
[5] Z. Ahmed, S. N. H. Kazmi, A. Malik, *Fitoterapia.* **1991**, *62*, 394.
[6] M. Hussain, D. N. Zahra, S. M. S. Hussain, E. Ahmad, I. Ahmad, A. Malik, Z. Ahmed, *Magn. Reson. Chem.* **2008**, *46*, 274.
[7] X. F. Zhang, P. T. Thuong, B. S. Min, T. M. Ngoc, T. M. Hung, I. S. Lee, M. K. Na, Y. H. Seong, K. S. Song, K. H. Bae, *J. Nat. Prod.* **2006**, *69*, 1370.
[8] C. J. Pouchert, J. Behnke, *The Aldrich Library of ^{13}C and ^{1}H FT-NMR spectra*, (1st edn), vol. 2, **1996**, p 1043.
[9] F. Jullien, B. Voirin, J. Bernillan, J. F. Bonvin, *Phytochemistry* **1984**, *23*, 12, 2972.
[10] L. Mitscher, S. R. Gollapudi, S. Drake, D. S. Oburn, *Phytochemistry* **1985**, *24*, 1481.
[11] M. Haruna, T. Koube, K. Ito, H. Murata, *Chem. Pham. Bull.* **1982**, *30*, 1525.
[12] M. Miyazawa, M. Hisama, *Biosci. Biotechnol. Biochem.* **2003**, *67*, 2091.

[13] M. Wang, J. E. Simon, I. F. Aviles, K. He, Q. Y. Zheng, Y. Tadmor, *J. Agric. Food Chem.* **2003**, *51*, 601.
[14] J. Shinoda, *J. Pharm. Soc. Jpn.* **1928**, *48*, 214.
[15] T. J. Mabry, K. R. Markham, M. B. Thomas, *The Systematic Identification of Flavonoids*, Springer: Heidelberg, **1970**, pp. 138, 140.
[16] T. J. Mabry, K. R. Markham, M. B. Thomas, in *The Systematic Identification of Flavonoids*, Springer-Verlag: New York, **1970**, pp 267.
[17] F. Imperato, P. Minutiello, *Phytochemistry* **1997**, *45*, 199.
[18] A. S. Awaad, D. J. Maitland, G. A. Seliman, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4624.
[19] C. Karl, G. Muller, P. A. Pedersen, *Phytochemistry* **1976**, *15*, 1084.
[20] S. Hara, H. Okabe, K. Mihashi, *Chem. Pharm. Bull.* **1987**, *35*, 501.
[21] X. L. Zhou, X. J. He, G. H. Wang, X. S. Yao, *J. Nat. Prod.* **2006**, *69*, 1158.

